The Effect of Gamma Radiation on Sprout Prevention and Its Physiological Mechanism in the Potato Tuber and the Onion Bulb

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The present study was carried out to confirm the sprout-inhibiting effect of gamma radiation from Co^{60} on potato tubers and onion bulbs at relatively low doses, and also to investigate the physiological changes following the treatment. Radiation levels of 3000, 7000 and 12000 r were employed for both materials.

I. Potato. "Irish Cobbler" potatoes were irradiated at the dormant period, on July 4, and at the beginning of sprouting period, on September 16.

(1) It was found that the very short sprouts appeared early in September on both control and treated tubers which had been irradiated in July, but subsequent elongation of the buds was completely inhibited in the tubers of 7000 and 12000 r lots. On the tubers of 3000 r lot, the growth of buds was more vigorous than those of the control.

(2) When the tubers had been irradiated in September, the development of tiny buds on the tubers of 12000 r lot was completely suppressed. Most tubers of 7000 r lot were also inhibited, but some of this lot commenced to develop their buds at the middle of January.

(3) Oxygen uptake in the tissue slices of all treated tubers increased immediately after the irradiation, then rapidly decreased to an extent as high as the control. But, the respiration of tubers which received the dose of 3000 and 7000 r in September, continued to increase, in parallel with the control tubers.

(4) There was a sharp increase of sugar content immediately after the irradiation, followed by the rapid decrease. After October, the remarkable increase appeared in the sprout inhibited tubers of 7000 and 12000 r lots.

(5) The concentration of ascorbic acid did not show any change by the irradiation.

II. Onion. "Senshu-nakadaka" onions were irradiated soon after the harvest, on June 28, and at the pre-sprouting period, on September 20.

(1) The sprouting of onion bulbs was completely inhibited even at the dose as low as 3000 r.

(2) Inner buds of onion bulbs which were compelled to inhibit the sprouting by the irradiation were browned and dead, but the injured parts did not extend to the outside of the buds.

(3) Consistent effect of irradiation on the respiratory activity was not observable in the disk parts, but, the oxygen uptake of inner bud seemed to be affected by the irradiation directly.

(4) The concentrations of sugar and ascorbic acid in the bulbs were not immediately affected by any dose of radiation used in this study.

(5) It was observed that the irradiation did not cause any important alteration on

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growth promoting and inhibiting substances in the inner buds.

INTRODUCTION

Although numerous investigations have been reported in recent years concerning the use of gamma radiation for the purpose of preservation of foodstaffs, rather disappointing results are prevalent from the practical point of view, because such irradiation often induce undesirable secondary effects such as the changes of flavor, color or taste. It is known that the inhibition of sprouting of potatoes is one of the successful applications of irradiation, but optimum doses of irradiation often varies with the investigators or conditions of potato. Sparrow and Christensen[12] reported that the sprouting was greately reduced at a dose of 5000 rep and completely inhibited at 20000 rep or higher. Brownell and coworkers[2], on the other hand, indicated doses of from 10000 to 15000 rep as minimums which were necessary for complete sprout inhibition. Irradiation dose of about 10000 r seems to be preferred by many investigators, notwithstanding Burton and Hannan[3], in recent work, reported that sprout suppression was succeeded at much lower doses such as 3500 to 8500 rep. Further, it was also observed that the tubers which received relatively high doses of gamma radiation increased the loss which was attributed to rotting during the storage. Schreiber and Highlands[10] found that the lots irradiated at high doses (28000-41000 rads) had 33% more rotting than the control and the low dose lots, in their experiment (14000-20500 rads), had 29% more rot than the control under commercial storage conditions. Duncan, Hooker and Heiligman[4], in the latest study, reported that the rot incidence of wounded, or inoculated tubers was progressively increased as irradiation intensity was increased, but non-wounded, non-inoculated tubers developed little storage rot at doses of from 2500 to 15000 rep.

With reference to the sprout inhibition of onion bulbs through the irradiation, available studies are very few if compared with the potato. Sawyer and Dallyn[9] found that the complete onion sprout suppression was obtained with 8000 rep.

The present study was carried out to confirm the effect of gamma radiation from Co^{60} on the inhibition of sprouting of onions and potatoes at relatively low doses and also to investigate the physiological changes following the treatment.

MATERIALS AND METHODS

"Irish Cobbler" potatoes, the main variety grown in Japan, had been harvested on June 16, and irradiated at the dormant period, on July 4, and at the beginning of sprouting period, on Setember 16, respectively. "Senshu-nakadaka" (Yellow Danvers Flat) onions had been harvested on June 20, then irradiated soon after the harvest, on June 28, and at the presprouting period (the period when the dormancy ended, *i.e.*, September 20). Both materials were stored on the shelf in a single layer, not piled up, at the room temperature throughout the experimental periods. Radiation levels of 3000, 7000 and 12000 r were employed.

Oxygen uptake and carbon dioxide evolution of potato tissue slices without the bud portions were measured by using Warburg apparatus at the temperature of 30°C. The respiratory activity of disk parts (bottom parts) and inner buds of onion bulbs were similarly measured.

The concentration of reducing and total sugars was determined by the Bertrand method.

Reducing and total ascorbic acid contents were analyzed by the titration method of 2, 6-dichlorophenol-indophenol.

Growth substances of inner buds in the irradiated onion bulbs, which received the dose of 10000 r on September 20, were determined according to the method reported by Tsukamoto and Asahira[13]. Condensed ether extracts of inner buds containing growth promoting and inhibiting substances were chromatographed. The chromatograms were divided into 10 equal parts, and the paper pieces were placed in sucrose solutions (3%, 2 ml) for 24 hours at 0°C. After that the Avena coleoptile sections of 3 mm were floated on each solutions for 20 hours at 20°C, then the length extension of the sections was measured.

RESULTS

Potato

1. Sprouting. It was found that very short sprouts (about 1mm) appeared early in September on both control and treated tubers which had been irradiat-

Fig. 1. Sprout-inhibited tuber which was irradiated at the dose of 7000 r and stored for 7 months, February 11.

ed soon after the harvest, but subsequent elongation of the buds was completely inhibited in the tubers of 7000 and 12000 r lots (Fig. 1). At the dose of 3000 r the growth of buds was more vigorous than the control (Fig. 2). When the tubers had been irradiated at the beginning of sprouting period (Septmber 16)

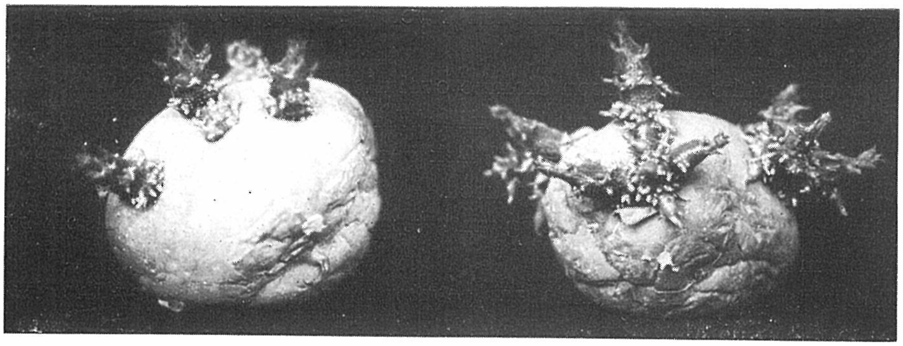

Fig. 2. Sprouted potato tubers, February 11. Left : Control Right : Tuber of 3000 r lot

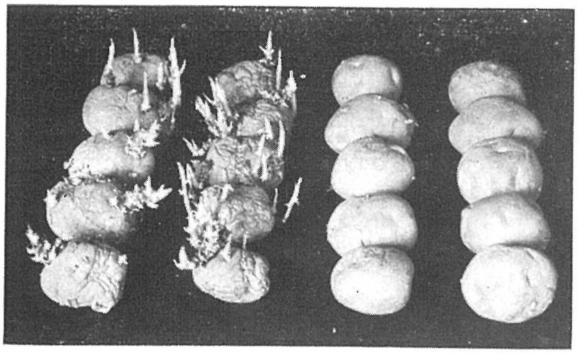

Fig. 3. Potato tubers stored in the darkness and 20°C for a month from the middle of January. From left to right control, 3000 r, 7000 r, and 1200 r lots.

the development of tiny buds on the tubers of 12000 r lot was completely suppressed by the treatment. In most tubers of 7000 r lot the growth of buds was also inhibited, but some of this lot commenced to develop buds at the middle of January, *i.e.*, 4 months later than the control. Tubers of 3000 r lot showed the same tendency with the early irradiated materials; the buds of treated potatoes being larger than that of the controls. Complete inhibition was observed even in darkness with favorable temperature for sprouting. Fig. 3

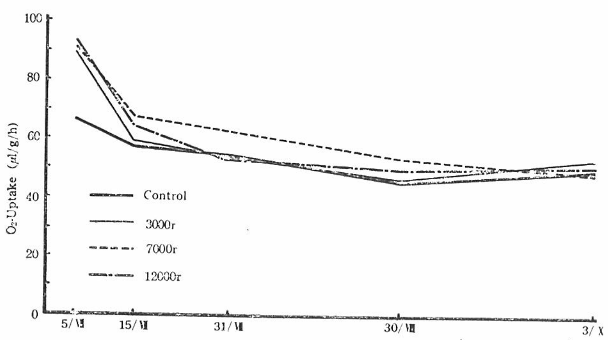

Fig. 4. Effect of gamma irradiation on the oxygen uptake of tissue slices of potato tubers (date of irradiation : July 4).

shows the tubers which were stored at 20°C in darkness for a month after the middle of January.

2. Respiration. When the tubers had been irradiated on July 4, oxygen uptake in tissue slices of all treated potatoes was 40% higher than the control on the day succeeding the treatment, then rapidly decreased to reach to an extent of untreated tubers (Fig. 4). In the tubers irradiated in September the similar spurt of oxygen uptake was also found, and the decreasing followed in the tubers of 12000 r lot. However, the oxygen uptake of tubers of 3000 and 7000 r lots continued to increase, in parallel with the control which was showing the gradual increase of respiration with the elongation of buds (Fig. 5).

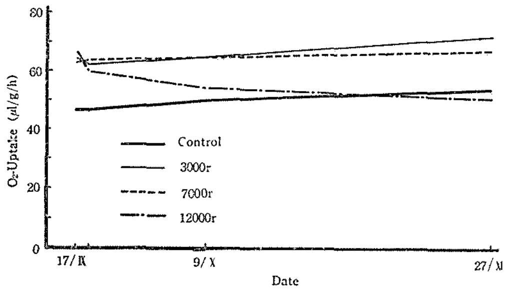

Fig. 5. Effect of gamma irradiation on the oxygen uptake of tissue slices of potato tubers (date of irradiation : September 16).

Though the same tendency was found in the carbon dioxide evolution of tissue slices, the increase immediately after the irradiation was more conspicuous, so the respiratory quotient was somewhat higher than the control in early period after the treatment, then settled down to a value nearly as high as the control. Brownell and coworkers[2] reported no significant effect of gamma radiation doses of 5000, 10000, and 15000 rep on tissue slices, but on the whole tubers, they found a drop of −30% in respiration rate during the first day after irradiation, followed by a sharp increase on the second day to about +60%, followed by a gradual decline to about −5% at the 13th week of storage. Thus the influence of irradiation on the respiratory activity of potato tubers was obviously recognizable, but it seemed that the change of respiration had no correlation with the mechanism of sprout inhibition directly because irradiation

Table 1. Carbon dioxide evolution of whole potato tubers at room temperature.

Experimental plots	Date of irradiation	
	July 4	Sept. 16
	mg/kg/h	
Control	13.2	13.6
3000 r	18.4	16.3
7000 r	8.8	7.7
12000 r	7.2	8.8

Date of experiment : February 4, 1959.

of 3000 r caused a marked change in respiration of tubers, while it had no sprout inhibiting effect. The rates of carbon dioxide evolution of the whole tubers were measured in February (Table 1). Sprouted tubers of control and 3000 r lot had higher evolution of carbon dioxide than sprout inhibited tubers as a matter of course. And the highest rate of evolution in the the tubers of 3000 r coincided well with the abundant growth of buds.

3. Sugar. Sugar contents increased immediately after the irradiation as in the case of respiration, the increase of sugar was mainly found in the non-reducing type much more than the reducing type (Fig. 6). The increased sugar concentrations rapidly decreased to an extent similar to the untreated tubers, then gradually decreased with the control. Pederson[7] also reported that after an initial decline the sugar concentration returned after 5 hours to the approximate original value, then followed by a significant increase with an apparent maximum after 24 hours.

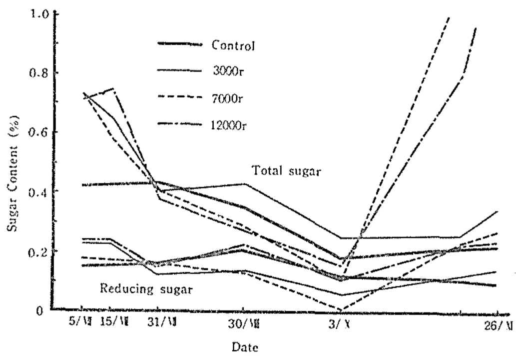

Fig. 6. Effect of gamma irradiation on the sugar content of potato tubers (date of irradiation : July 4).

After October, the remarkable increase appeared in the sprout-inhibited tubers of 7000 and 12000 r lots, the total sugar contents were 4–5 times as much as the tubers of control and 3000 r lots at the end of November, while these sprouted tubers also showed some increase during the period. Reducing sugar contents of sprout-inhibited tubers were about 3 times higher than the control at the same period. It was supposed that the accumulation of sugar which was derived from the degradation of starch due to lowering of storage temperature, must have occurred in the sprout-inhibited tubers, but in the tubers of control and 3000 r lots the sugar must have been consumed for the elongation of buds.

4. Ascorbic acid. There was no significant difference of ascorbic acid concentration between the control and the irradiated tubers throughout the storage period from the day succeeding the irradiation (Fig. 7). Losses of ascorbic acid by irradiation have been reproted by some authors. Sereno[11], for instacne, indicated an initial loss of ascorbic acid immediately after irradiation, followed by an arresting of this loss, and later by a gradual recovery of the vitamin. But in practical dose of irradiation, the loss of ascorbic acid may be a matter of out of the question.

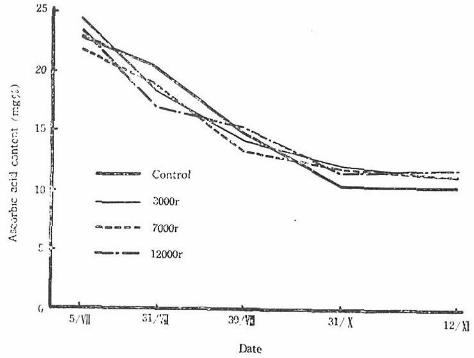

Fig. 7. Effect of gamma irradiation on the concentration of *l*-ascorbic acid in the potato tubers (date of irradiation : July 4).

5. Undesirable changes. The increase of reducing sugar content in the irradiated tubers during the storage is considered as deterioration of quality because these tubers produce browned potato chips. However, it dose not matter as potato is little utilized in that form in this country. Very little incidence of storage rot was met with in any experimental lots. Changes of color or flavor in treated tubers also did not appeare at any doses of irradiation used in this study.

Onion

1. Sprouting. The sprouting of onion bulbs was completely inhibited even at the dose as low as 3000 r irrespective of treated dates. When the irradiation was conducted on July 28, there was no change of internal aspects in treated bulbs for 2 months after the treatment. It was found, however, at the end of September, the inner buds of treated bulbs were browned and dead without exception (Fig. 8). The browned parts did not extend to the outside

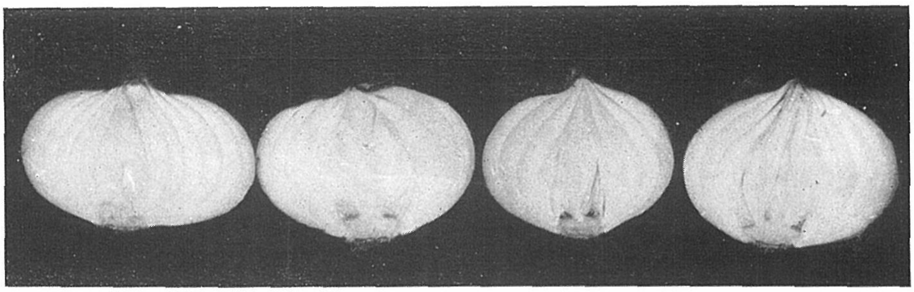

Fig. 8. Sections of onion bulbs on September 30 (date of irradiation : June 28), showing the browned inner buds in the irradiated bulbs. From left to right control, 3000 r, 7000 r, and 1200 r lots.

of the buds after that. In the bulbs irradiated in September, the appearance of browning was considerably rapid, and it was already evident 3 weeks after the irradiation. Figure 9 shows the scaly leaf development which took place during the storage period (by then yet unsprouted bulb in the lot of control was compared with the bulb of 7000 r lot). It was obviously recognizable that the growth of inner buds had been ceased by the irradiation, while in the control bulbs, the scaly leaves had elongated and increased the number during the storage. Aoba[1] indicated in "Imai-wase" onions that the increase of the number of scales and elongation of scaly leaves in stored onion was evidently observable early in September. These results given above suggest that the browning (the death of inner buds) would have occurred when or after inner buds commenced to elongate and/or to increase the number. In the bulbs which had been irradiated on September 20, the browned parts were somewhat larger than that of the bulbs treated soon after the harvest. It means that the growth of inner buds might have occurred during the period, so the irradiation in the earlier stages would be preferable for practical use than later stages.

Undesirable changes were found in the treated onion bulbs when they were stored until February-March (8-9 months after the harvest); a scaly leaf adjacent to the injured inner bud elongated and curved within the bulbs (Fig. 10), and empty gaps appeared between scaly leaves. It can be assumed, there-

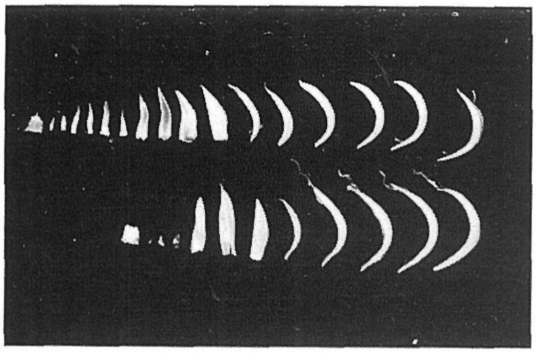

Fig. 9. Disjojnted scaly leaves on December 10. Upper ; Control bulb showing the increase of number and the elongation of inner buds. Lower : Irradiated bulb at the dose of 12000 r in June. Left end one of each row is the disk part and the innermost bud.

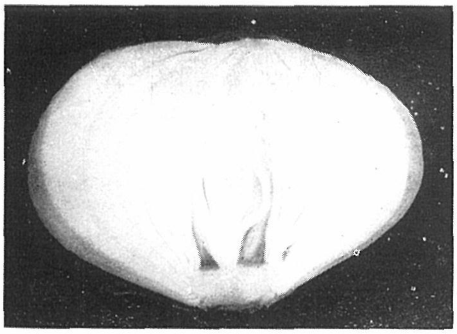

Fig. 10. A section of treated buld on February 3 (irradiation : 7000 r, June 28).

fore, from the commercial basis that the prolonged storage life of irradiated onion bulbs seemed to be 4-5 months over ordinary storage period.

2. Respiration. Respiratory activity was measured mainly on the disk part which seems to be physiologically important parts of onion bulbs[6]. Measurements were also taken to some extent from the inner buds. No consistent irradiation effect was found on the oxygen uptake of disk part, but, when the control bulbs had increased the respiration after the pre-sprouting period, the increase of it in the treated bulbs was considerably suppressed (Fig. 11).

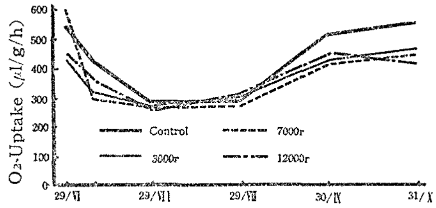

Fig. 11. Effect of gamma irradiation on the oxygen uptake of disk parts of onion bulbs (date of irradiation : June 28).

There was no difference of R.Q. in disk part between the treated bulb and the control during the dormant period. After the pre-sprouting period, R.Q. of all treated bulbs was apparently lower than the control, and the latter maintained a uniform value of about 1. The respiration of inner buds seemed to be influenced directly by the irradiation. When the bulbs had been irradiated in September, oxygen uptake of inner buds of 7000 and 12000 r lots was 20-30% lower than the control 9 days after the treatment. In other experiments, however, authors observed that irradiation of 10000 r caused remarkable increase of oxygen uptake of inner buds (about twice as much as the control 3 days after treatment), followed by a rapid decline to an extent of control bulb, but did not fall lower than the control.

3. Sugar and ascorbic acid. Sugar contents of bulbs were not immediately affected by any dose of radiation used in this study. However, at the time when the non-reducing sugar content in the untreated bulbs gradually decreasing after the bulbs had commenced sprouting, the contents in the treated bulbs were not affected for any change (Fig. 12).

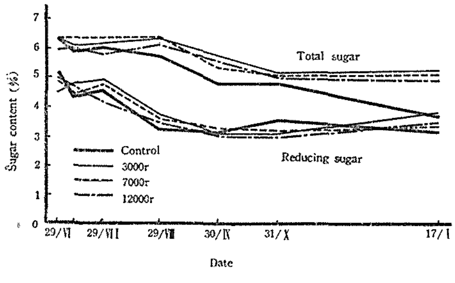

Fig. 12. Effect of gamma irradiation on the sugar contents of onion bulbs (date of irradiation : June 28).

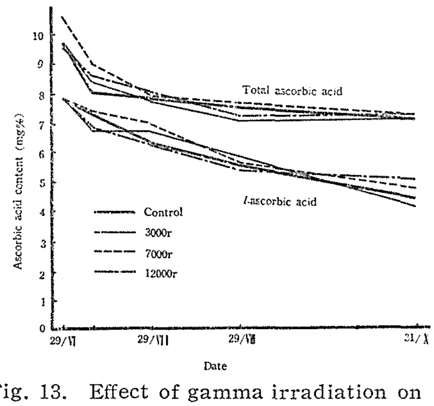

Fig. 13. Effect of gamma irradiation on the ascorbic acid contents of onion bulbs (date of irradiation : June 28).

Ascorbic acid contents in treated bulbs also did not show any difference from the control: It was gradually decreasing during the storage (Fig. 13).

4. Growth substance. Materials used in this experiment was different from the above-mentioned samples in the place of production, though belonging to the same variety, and they were irradiated at the dose of 10000 r on September 20. Growth substances of inner buds were bio-assayed 5 days after the treatment, when no visible changes as browning appeared in the inner buds. The results of the experiments have been plotted as histograms (Fig. 14, 15). The horizontal lines on the figures represent the extension of coleoptiles in the sucrose solution not containing the growth substnces, so that the coleoptile extensions greater than this limit represent growth promotion and those below represent inhibition.

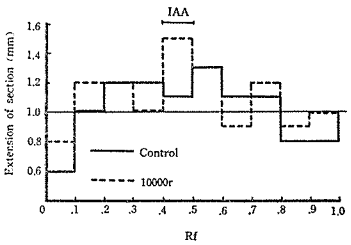

Fig. 14. Effect of gamma irradiation on the growth substances of inner buds of onion bulbs (acidic fractions of ether extracts).

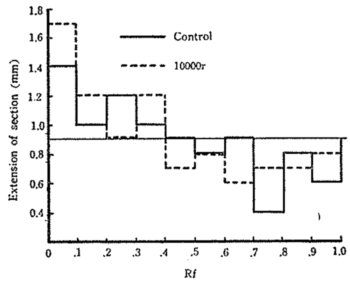

Fig. 15. Effect of gamma irradiation on the growth substances of inner buds of onion bulbs (neutral fractions of ether extracts).

In the acidic fractions of ether extracts, the existence of growth-active substance was obviously observed with Rf of about 0.5, which corresponds to indoleacetic acid, in both control and irradiated materials. Another growth promotor with lower Rf, and inhibitors with higher Rf and with the lowest Rf were also recognized to exist. Moreover, the distribution and amounts of these growth substances had relatively small differences between control and treated bulbs. In the neutral fractions, which exhibit the growth promotor at lower Rf and the inhibitor at higher Rf, similar tendency was obtained. In any case, it seemed that the irradiation did not cause any important alteration on growth substances.

5. Sensory changes. Significant alterations of characteristic flavor, taste, and color of onion bulbs by the irradiation were not found throughout the experimental period except the browning of inner buds.

DISCUSSION

It was found that the sensibility to gamma radiation considerably differed between potato tubers and onion bulbs: At the dose of 3000 r, the sprouting of onion bulbs was completely suppressed, while in potatoes, greater and somewhat abnormal buds appeared on the treated tubers. Further, the mechanism of sprout inhibition also seemed to differ between both species within the used doses of irradiation: Inner buds were browned and dead in onion bulbs, but potato buds did not show any visible changes in the tubers in which the growth of buds were completely suppressed. In many articles, it has been reported that the death of cell by irradiation do not take place immediately after the treatment, not giving direct response to kill the cell, but it contributes to leave effect to the next stage of cell activity to multiply. Immediate killing of cells without waiting cell division stage, may be followed by very much higher doses which are almost impossible to apply, practically. Such facts may suggest a certain change in chromosome or in genetical behavior occur by the irradiation, the effect of which appearing later at the next cell dividing stage: Actual killing may occur either during or after the cell division. When the onion bulbs had been irradiated on June 28, the browning of inner buds was found in September (when the growth of inner buds had commenced) but in the bulbs irradiated in September, the browning appeared within 3 weeks after the treatment. This fact may explain that the death of inner buds in onion bulbs would have been caused through a above-suggested reason.

In potatoes, the recovery of growing ability of buds was found in some tubers which had been irradiated at the dose of 7000 r in September. In this case, it is possible to consider that some indispensable substances which had been lost by the irradiation were regained, or the inhibitor of cell division which had been produced by the irradiation disappeared during the period. If it is the case, a complete suppression of bud elongation would be the result when such function was not enough.

In potato tubers, immediately after the irradiation, it was found that both respiration rate and sugar content increased at the same time. Pederson[7] observed the initial decrease in sugar content (during the first few hours) and the following rapid increase, but he did not measure the respiration. Suppose that the metabolism of the potato tubers, he assumes, is markedly increased either immediately or shortly after irradiation, there should follow a corresponding increased requirement for sugars with the result that the prevailing sugar concentration decreases. After a few hours, the equilibrium of starch $\rightleftarrows$ sugar is altered due to the increased requirement for sugar by the potato and consequently, in order to reestablish the equilibrium, the potato responds by increasing the enzymatic breakdwon of starch into sugars. The results obtained in our experiment support this Pederson's assumption.

It seemed that the growth substance had no direct correlation to the mechanism of sprout inhibition. Inner buds of irradiated onion bulbs contained both growth promotor and inhibitor to the same degree as the control. Brownell and coworkers[2] found in the tubers of "Pontiac" potatoes that the gamma radiation increased the hormone concentration and decreased the inhibitor, and they observed in "Sebago" tubers no significant difference to exist between control and irradiated tubers.

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